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MEWBURN ELLIS
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(57) Novel compositions are provided comprising a conjugate of a physiologically active compound and a receptor specific for a red cell, platelet or endothelial cell. The binding of the conjugate to the aforementioned cells provides for prolonged half-lives of the physiologically active compounds, providing for enhanced immune response in the production of vaccines, prolonged therapeutic dosages, or prolonged activation or inhibition of cellular activity, where the circulating dosage of a drug may be maintained at a desired level.

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Technical Field

The field of this invention is drug delivery systems.

Background

The administration of drugs provides many challenges. Depending upon the nature of the drug, the drug may be directed preferentially to different sites in the body, may be cleared more or less rapidly, may bind to a variety of proteins, may be subject to degradation and catabolism, renal clearance, or the like. While in some cases, there is an interest in having rapid clearance of the drug, in most situations, it is desired to have a therapeutic blood concentration over long periods of time.

In order to maintain the blood level at or above the therapeutic concentration, drugs are frequently administered at dosages substantially above the therapeutic dosage or administered incrementally at levels below the therapeutic dosage at repetitive time intervals, or administered repeatedly for long periods of time to maintain the therapeutic blood concentration until the therapeutic dosage is achieved. Neither of these situations is particularly attractive, since in most cases the drug will have side effects, so that the side effects are potentiated at the elevated drug dosages. Furthermore, where the drug is administered sequentially, until a therapeutic dosage is achieved, the patient is not benefiting from the presence of the drug, so that there may be an extended time period between the time when drug administration begins and the time when the patient is obtaining some benefit from the drug. Also, frequent administration of a drug, especially by parenteral administration, implies a high level of health care, higher cost, and lower comfort for the patient.

A wide variety of techniques have been suggested to improve modes of drug administration. One technique involves therapeutic dosage monitoring, where a large bolus of the drug is initially given to bring the patient rapidly to the therapeutic dosage level and the level of drug in the bloodstream is monitored, so as to ensure that the dosage is maintained at an adequate effective dosage. Quite clearly, this is expensive in requiring repetitive sampling and monitoring of drug levels in the host. This is only feasible with those drugs, where chronic administration is provided and the drug is monitored at relatively extended intervals. Other techniques have involved slow release capsules or particles, where the capsules or particles continuously release drug. The particles create numerous problems, being ingested by macrophages, being subject to rapid clearance, and being difficult to regulate.

There is, therefore, substantial interest in being able to provide for improved methods of administering drugs, where the drug will have an extended half-life in the patient, may be relatively uniformly distributed throughout the vascular and/or lymphatic system or directed to a particular site and allow for ease of administration.

SUMMARY OF THE INVENTION

Physiologically active compounds are administered conjugated to receptors which bind to surface membrane proteins of erythrocytes, platelets or endothelial cells, where in the case of erythrocytes and platelets, the conjugates may be administered bound to the cells. Specifically, the compound, usually a drug, is conjugated to a monoclonal antibody or fragment, desirably with a cleavable linkage, where the antibody fragment thereof is specific for a target epitope of the indicated cells. The conjugates are conveniently administered parenterally.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

Methods and compositions are provided for the administration in vivo to mammalian hosts, particularly primate hosts, of a wide variety of physiologically active compounds, where prolonged availability of the compound to be achieved. The method employs a conjugate of the compound, usually a drug, with a receptor having a high affinity for erythrocytes, platelets or endothelial cells, where the site of conjugation is at other than the binding site so as to retain binding affinity. Depending upon the purpose of the drug and its activity, the drug may be linked to the receptor by a cleavable or non-cleavable linkage, where by cleavable is intended a linkage which is cleaved at a desired rate for release of the drug from the receptor under physiological conditions to provide a physiologically effective dosage of the drug.

The receptor will, for the most part, be a monoclonal antibody or fragment thereof, which is specific for an epitope substantially specific for an erythrocyte, platelet or endothelial cell.

The antibody or fragment thereof may be any one of the subtypes or isotypes, only being one that does not participate in complement effected lysis. Therefore, the whole antibody may be IgA, IgD, IgG₁ or IgG₄ or corresponding isotypes of other species, while fragments may be from any isotype. Normally, the antibody will be one which is allogenic, although in some instances, xenogenic antibodies may be employed. Particularly, xenogenic antibodies may be employed in those situations where the host is immunocompromised,

or the antibody or antibody fragment is non-antigenic, despite its being xenogeneic, or one wishes to enhance the antigenicity of the physiologically active compound.

The whole antibody need not be used, fragments being frequently useful, such as F_v , Fab, $F(ab')_2$, the heavy chain, a single-chain antigen binding protein, or the like. The fragment which is used, should retain at least a substantial portion, better than about fifty percent (50%) of the original affinity of the antibody.

The binding site of the antibody should be relatively specific for the target cell. That is, better than about fifty percent (50%), preferably more than about seventy-five percent (75%) of the antibody introduced to the host, which binds to antigens will bind to the target cell. In this way, one can assure that a major proportion of the conjugate will be present over extended periods of time and will not be endocytosed, cleared from the blood stream, or the like.

Monoclonal antibodies may be obtained in accordance with conventional ways. For example, for monoclonal antibodies specific for erythrocytes, lymphocytes may be collected from an individual with a positive direct Coombs test, particularly of the IgG type, (without complement) without a showing of hemolysis. The lymphocytes may be immortalized by any convenient means, e.g. Epstein-Barr virus transformation, followed by screening for antibodies having the desired specificity and affinity, as well as the desired isotype. The antibodies may then be modified in a variety of ways, by enzymatic cleavage to provide for the fragments, using papain, chymotrypsin, pepsin, reduction with cleavage of intramolecular disulfide linkages or the like.

The monoclonal antibodies may be from any source, such as primate, particularly human, murine, lagomorpha, canine, ovine, porcine, equine, or the like.

Alternatively, polyclonal antibodies raised against a fragment of the target antigen, such as a synthetic peptide, may be used.

Antibodies which bind to erythrocytes may be further characterized by binding to red cells, particularly O Rh negative red cells, and to panels of red cells of known phenotypes. Those cells which react with all the cells of the panel and, furthermore, show negative results with the direct Coombs test using anti-complement globulin are selected. Ref: Stratton, F.; Rawlinson, Vi; Merry, A.H.; Thomson, E.E.; *Clin. Lab. Haematol.* 1983, 5:17-21. In addition, the antibodies are screened as to cross-reactivity with other cell types, such as lymphocytes, myelomonocytes, and the like. Similarly, for antibodies specific for platelets or endothelial cells, the antibodies are selected to be

specific for such cells and not for other cells which may be encountered in the host. For the most part, with erythrocytes the number of conjugates will be below the level that causes hemolysis, usually fewer than about 5×10^3 /cell.

In some situations activation of the target cell will result in up-regulation of a surface membrane protein. For endothelial Cells, addressins, e.g. ELAM-1, or integrins, e.g. VLA-4, may be up-regulated in the case of inflammation. In this way, the drug may be localized as the site of inflammation, providing for a localized concentration at the target region.

The cells which are selected have a number of advantages. These cells are present in high number in the vascular system. Platelets are present in from about $1-4 \times 10^5$ / μ l, while erythrocytes are present in about $4-6 \times 10^6$ / μ l. The cells have a long half-life and by appropriate choice of epitope, endocytosis is avoided. The red cells and platelets lack a nucleus and cell division capability. All of the cells have a wide distribution in capillaries and tissue and express specific epitopes associated with their specific differentiation. Furthermore, for the erythrocytes and platelets, those cells may be readily collected, combined with the conjugate, and administered to the host. The cells may be autologous or allogenic.

If desired, the antibodies can be prepared or modified in a variety of ways. Chimeric antibodies may be prepared, where the constant region may be modified as to isotype or species. For example, murine monoclonal antibodies may be prepared, the genes encoding the heavy and light chains isolated, and the constant regions of the heavy and light chains substituted with the appropriate constant regions of human constant regions to provide for a chimeric antibody which lacks the antigenicity of the murine constant region. Alternatively, variable regions obtained from a host may be cloned and mutated and then screened to identify specific binding affinities. A further alternative is to exchange not only the constant region of the heavy and light chain, but also exchange the framework regions of the variable domains, so as to further reduce the antigenicity of the antibody. Numerous techniques are described in the literature, for example, "The synthesis and *in vivo* assembly of functional antibodies in yeast," Wood, CR., Boss, M.A., Kenten, J.H., Calvert, J.E., Roberts, N.A., Emstage, J.S., *Nature*, Apr 4-10, 1985, 314(6010):446-9; "Construction of chimaeric processed immunoglobulin genes containing mouse variable and human constant region sequences," Takeda, S., Naito, T., Hama, K., Noma, T., Honjo, T., *Nature*, Apr 4-10, 1985, 314(6010):452-4; "A recombinant immunotoxin consisting of two antibody variable domains fused to *Pseudomonas* exotoxin," Chaud-

hary, V.K., Queen, C., Junghns, R.P., Waldmann, T.A., FitzGerald, D.J., Pastan, I., Laboratory of Molecular Biology, DCBD, National Cancer institute, Bethesda, Maryland 20892, *Nature*, Jun 1, 1989, 339(6223):394-7; "Binding activities of a repertoire of single immunoglobulin variable domains secreted from *Escherichia coli* [see comments]," Ward, E.S., Gussow, D., Griffiths, A.D., Jones, P.T., Winter, G., MRC Laboratory of Molecular Biology, Cambridge, UK, *Nature (ENGLAND)*, Oct 12, 1989, 341(6242):544-6, ISSN 0028-0836, Comment in *Nature*, 1989, Oct 12, 341(6242):484-5.

The physiologically active compounds which are conjugated to the receptor will vary widely depending upon the nature and the purpose of the physiologically active compound. In the case of vaccines, the physiologically active compound may be an antigen or immunologically active fragment thereof associated with a pathogen, e.g., virus, bacteria, protozoa, fungus; an allergen, or other parasite or debilitating antigen or an histocompatibility antigen to enhance antigen presentation. In this situation, the physiologically active compound may be bound to the receptor by a cleavable or non-cleavable linkage, depending upon the desired level of the antigen in the blood stream over time. By providing for an antigen which binds to the cells, the lifetime of the antigen in the blood stream may be greatly extended, so as to continuously stimulate the immune system over an extended period of time.

Other physiologically active compounds may be associated with agonist or antagonist activity. Thus, a wide variety of hormones, cytokines, surface membrane protein receptors, synthetic drugs, enzyme inhibitors, cytotoxic compounds, antibodies, T-cell receptors, active peptides, homing receptors, prostaglandins, catalytic antibodies, or the like, or physiologically active fragments thereof may be conjugated to the subject receptor. Illustrative compounds may include growth factors, e.g., colony stimulating factors, including M-, G-, G.M-; interleukins, 1-10, interferons, epidermal growth factor, fibroblast growth factor, platelet-derived growth factor, transforming growth factor- α , or - β , neuroactive peptides, tumor necrosis factor, etc., or corresponding cell receptors; insulin, somatomedin, somatostatin, follicular growth hormone, ACTH, VIP, antibiotics such as penicillin, cephalosporin, neomycin, aminoglycosides, quinolones, sulfamides, tetracyclines, sulfones, quinine; antibodies to interleukins, TNF, rheumatoid factors, etc.; vitamins, anti-coagulants, or the like.

The nature of the linkage between the physiologically active compound and the receptor will vary widely depending upon the nature of the physiologically active compound, whether one wishes to retain it bound to the receptor or allow

for its release into solution, and the rate at which one wishes to provide for the release. A wide variety of linkages will be unstable under physiological conditions, where the instability may be as a result of enzymatic or non-enzymatic reactions. For example, ester linkages can be provided which are relatively labile and will be cleaved over time in the blood stream. By providing for varying degrees of steric hindrance, different degrees of lability can be achieved. Alternatively, sequences can be provided, which are recognized by a wide variety of proteases. For example, a series of arginine-lysine dimeric units will be sensitive to trypsin. Other protease labile linkages may be employed, where the protease may be found in the bloodstream. Alternatively, one may employ disulfide linkages, which will be subject to reductive cleavage. Other functionalities which may find use include oligosaccharides, thiol eaters, nucleic acids, or the like. (By "stable" is intended that the linkage will not be preferentially cleaved as compared to other bonds in the molecule, "unstable" intends preferential cleavage.)

There are extensive reports in the literature of procedures for covalently joining a wide variety of compounds to prepare conjugates with different functional groups. Various synthetic schemes may be envisioned, depending upon the nature of the physiologically active compound. Thus, where sulfhydryl groups are present, these may be readily activated to provide for linkage to a sulfhydryl group to provide a disulfide. Carboxyl groups may be readily activated with a wide variety of hydroxyl compounds or carbodiimide, where the ester or anhydride is allowed to react with hydroxyl or amino groups to provide esters or amides. Other linkages may also find application, such as imines, hydrazines, etc.

The antibody fragment and the active compound may be genetically engineered as a single construct, cloned and expressed using a variety of vectors and host cells. Alternatively, both can be chemically synthesized.

Depending on the nature of the physiologically active compound, on the average, less than one, or one or more molecules of the physiologically active compound may be conjugated to the receptor. In some instances, it may be desirable to modify the receptor so as to provide for a target functionality which may be used to link the physiologically active compound. For example, one may introduce a maleimide group, where a thiol may bind to the maleimide to provide for a thioether. By employing a disulfide, one will then provide for a disulfide linkage.

The subject compositions may be administered in a wide variety of ways. If desired, the conjugate may be first bound to the cells in appropriate

proportion followed by administration of the conjugate-bound cells to the host. Alternatively, the conjugate may be administered to the host for binding to the target cells. The amount of the conjugate which is administered will vary widely, depending upon the nature of the conjugate, the purpose for the conjugate, the therapeutic dosage, the physiological activity of the compound when present as a conjugate, and when bound to a cell, and the like. Therefore, the amount of conjugate administered to a host may vary from 1 pg to 10 mg/kg of host.

The subject compositions will, for the most part, be administered parenterally, such as intravascularly, intraarterially, subcutaneously, or the like. For the most part, physiologically active carriers will be employed, such as water, saline, phosphate buffered saline, aqueous ethanol, plasma, proteinaceous solutions, glucose solutions, or the like. The concentration of the conjugate will vary widely, generally ranging from about 1 pg/ml to 10 mg/ml. Other additives which may be included include buffers, where the media are generally buffered at a pH in the range of about 5 to 10, where the buffer will generally range in concentration from about 50 to 250 mM, salt, where the concentration of salt will generally range from about 5 to 500 mM, physiologically acceptable stabilizers, and the like.

The subject compositions may be used for the treatment of a wide variety of diseases. For synthetic drugs, compounds which may find use include barbiturates, theophylline, dilantin, etc. For treatment of chronic infection, various antibiotics may be used. For modulating the immune system, various cytokine or corresponding receptors may be employed, particularly receptors IL-2 or IL-4 or antibodies to the interleukins or their receptors. In the case of virulent infections, where the virus is trophic for a particular surface membrane protein, surface membrane proteins may be employed, such as CD-4 in the case of HIV virus. For vaccines, the physiologically active compound may be any protein of the pathogen which results in neutralizing antibodies, such as envelope proteins, capsid proteins, surface membrane proteins, cell wall proteins and saccharides, and the like.

The following examples are offered by way of illustration and the way of implementation.

EXPERIMENTAL

Human monoclonal antibodies were derived from an EBV transformation of lymphocytes collected from an individual with a positive direct Coombs test. This individual had a positive direct Coombs test of the IgG type (without complement) and showed no sign of hemolysis. Anti-red cell

antibodies were selected by liquid phase ELISA and indirect Coombs test using O Rh negative red cells, and further characterized using panels of red cells of known phenotypes. Only monoclonal antibodies reacting with all cells of the panels and showing negative results with the indirect Coombs test using anti-complement globulin were selected. Cell cross-reactivity was further tested by a cytofluorometric method against various cell types (T and B cells, platelets, monocytes, polynuclear cells) to check for non-cross reactivity. Monoclonal antibody isotype was tested by ELISA using monospecific antibodies. Briefly, monoclonal antibody specimens were incubated in microtiter plate wells coated respectively with anti-IgG₁, -IgG₂, -IgG₃, -IgG₄, -IgM, -IgA goat specific antibodies, after washing and incubation with an anti-human immunoglobulin goat antibody peroxidase conjugate and washing. Color development following addition of o-phenylene diamine was measured spectrophotometrically at 495 nm.

Several anti-red blood cell monoclonal antibodies (IgG₄) were selected: CHP1, CHP2 and CHP3. Fab and F(ab')₂ fragments were made.

The subject antibody (CHP2) is conjugated to recombinant purified HBsAg using p-maleimidobenzoic acid. The acid is activated with carbodiimide and combined with HBsAg in stoichiometric ratio. The product is then reacted directly with Fab to provide the conjugate, which is then purified on HPLC. The subject conjugate is then administered to a mammalian host to induce an immune response to HBsAg.

A 5 mg dose of purified, sterile, conjugate was administered intravenously to one chimpanzee (C23). Control chimpanzees were as follows: C24 was injected with an equivalent dose of antibody fragment conjugated to bovine serum albumin; C25 was vaccinated using the Hevac B vaccine (Pasteur Vaccin) according to the manufacturer's instructions; C26 received a 5 mg dose of irrelevant human IgG₄ antibody fragment conjugated to HBs using the same conjugation, purification and administration procedures as for the conjugate administered to C23. The following parameters were monitored before and weekly (for 16 weeks) after injection:

Hemolysis was monitored by:

- red blood cell count
- hemoglobin blood level
- hematocrit
- reticulocyte count (using a Coulter instrument)
- bilirubin serum level

Binding of antibodies, conjugate and complement to red blood cells was monitored by modification of the standard direct Coombs test as follows:

- Direct Coombs test, *Fab type* (using an anti-human IgG, Fab fragment specific anti-globulin)
- Direct Coombs test, *Complement type* - (using an anti-human Complement specific anti-globulin)
- Direct Coombs test, *Fc type* (using an anti-human IgG, Fc fragment specific anti-globulin)
- Direct Coombs test, *HBs type* (using a goat anti-HBs specific anti-globulin)

Titration of Anti-HBs antibodies was performed by solid phase ELISA (Abbott Laboratories).

Results

None of the chimpanzees showed any biological evidence of pathological hemolysis. C23 developed immediately after the conjugate injection a positive, Fab type, and HBs type, direct Coombs test, which became negative at week 8. C24 developed immediately after the conjugate injection a positive Fab type only (HBs type negative) direct Coombs test, which became negative at week 9. The direct Coombs test of the Fc and Complement type became weakly positive at week 7 (until week 9) for C23 and C24, probably reflecting the anti-Human Fab and anti-HBs (C23) or the anti-Human Fab and anti-BSA (C24) antibody response. The direct Coombs test remained negative for C25 and C26.

Anti-HBs antibodies were detected in C23 at week 3 (1:200) with a peak end point titer of 1:3200 at week 17. Anti-HBs antibodies were detected in C26 at week 4 (1:100) and remained between 1:50 and 1:200 subsequently. No anti-HBs antibodies were detected in C24. Anti-HBs antibodies were detected in C25 at week 4, with a peak end-point titer of 1:6400 at week 15, 3 weeks after the third monthly injection.

It is evident from the above results, that the subject methodology provides for prolongation of the presence of a physiologically active compound in a mammalian host. In this manner, greatly enhanced immune responses or prolonged treatment with a physiologically active compound may be achieved. In this way, the circulation dosage of a compound may be controlled, while maintaining a therapeutic dosage over an extended period of time.

All publications and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

Claims

1. A method for administering to a mammalian host a physiologically active compound in vivo with an extended half-life, said method comprising:
administering said compound conjugated to a ligand or fragment thereof specific for a receptor of an endothelial cell, erythrocyte or platelet, wherein said receptor is substantially absent on other cell types.
2. A method according to Claim 1, wherein said ligand is specific for an erythrocyte.
3. A method according to Claim 2, wherein said conjugated compound is administered bound to an erythrocyte.
4. A method according to Claim 1, wherein said compound is conjugated through a linkage labile under physiological conditions.
5. A method according to Claim 1, wherein said compound is conjugated through a linkage stable under physiological conditions.
6. A method for administering to a mammalian host a physiologically active compound in vivo with an extended half-life, said method comprising:
administering said compound stably covalently conjugated to a ligand specific for a receptor of an endothelial cell, erythrocyte or platelet, wherein said receptor is substantially absent on other cell types, and said compound is an antigen of a pathogen or immunologically active fragment thereof.
7. A method for administering to a mammalian host a physiologically active compound in vivo with an extended half-life, said method comprising:
administering said compound covalently conjugated to a ligand specific for a receptor of an endothelial cell, erythrocyte or platelet, wherein said receptor is substantially absent on other cell types, said compound is conjugated through a linkage labile under physiological conditions, and said compound is a drug.

8. A compound comprising a monoclonal antibody or fragment thereof covalently conjugated
et other than the binding site of said antibody
or fragment thereof with a physiologically ac- 5
tive compound, wherein said antibody or frag-
ment thereof is specific for an epitope substan-
tially specific for on endothelial cell, eryth-
rocyte or platelet.
9. A compound according to Claim 8, wherein 10
said cell is an endothelial cell.
10. A compound according to Claim 8, wherein
said cell is an erythrocyte. 15
11. A compound according to Claim 8, wherein
said cell is a platelet.
12. A compound according to Claim 8, wherein
said compound is an antigen of a pathogen or 20
immunologically active fragment thereof.
13. A compound according to Claim 8, wherein
said compound is a drug. 25
14. A compound according to Claim 13, wherein
said drug is a naturally occurring compound or
physiologically active fragment thereof.
15. A compound according to Claim 8, wherein 30
said antibody is IgG.

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PARTIAL EUROPEAN SEARCH REPORT

Application Number

which under Rule 45 of the European Patent Convention
shall be considered, for the purposes of subsequent
proceedings, as the European search report

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DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 5)
X	WO-A-8 601 409 (DANA-FARBER CANCER INSTITUTE) 13 March 1986 * page 7, line 23 - line 34; claims 1,15-25 *	1,4-8, 12-14	A61K47/48
X	WO-A-9 011 783 (CENTOCOR INC.) 18 October 1990 * page 3, line 2 - line 15 * * page 3, line 30 - page 4, line 13; claims *	1,4-8, 11-15	
X,Y	WO-A-9 012 585 (ONCOGEN LTD PATRNSHIP) 1 November 1990 * page 17, line 14 - line 28; claims *	1-15	
X,Y	EP-A-0 066 296 (EISAI CO.) 8 December 1982 * claims 2,3,4,5; example 3 *	1-15	
-/--			TECHNICAL FIELDS SEARCHED (Int. Cl. 5)
			A61K

INCOMPLETE SEARCH

The Search Division considers that the present European patent application does not comply with
the provisions of the European Patent Convention to such an extent that it is not possible to carry
out a meaningful search into the state of the art on the basis of some of the claims

Claims searched completely :

Claims searched incompletely :

Claims not searched :

Reason for the limitation of the search:

see sheet C

Place of search

THE HAGUE

Date of completion of the search

15 JULY 1993

Examiner

BERTE M.J.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another
document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or
after the filing date
D : document cited in the application
L : document cited for other reasons
.....
A : member of the same patent family, corresponding
document

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DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. Cl. 5)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
X	STN FILE SUPPLIER & FILE MEDLINE AN=8828853 (KARLSR.) & CLIN. EXP. IMMUNOL. vol. 72, no. 1, April 1988, pages 164 - 167 S. RATH ET AL. 'IGC SUBCLASS COMPOSITION OF Ab TO HBSAG IN CIRCULATING IMMUNE COMPLEXES FROM PATIENTS WITH HEPATITIS B VIRUS INFECTIONS.' * the whole document *	1,12,15	
X	STN FILE SUPPLIER & FILE BIOSIS AN=86:142723 (KARLSR.)	1-3,6-8, 10,12-15	
X	IMMUNOL. CLIN. SPER. vol. 4, no. 3, 1985, pages 177 - 188 (A. CRAXI ET AL. 'IN-VITRO CYTOTOXICITY TO HEPATITIS B SURFACE ANTIGEN-COATED AUTOLOGOUS TARGET CELLS IN HEPATITIS B VIRUS INFECTION.' * the whole document *	1-2,4, 6-8,10	TECHNICAL FIELDS SEARCHED (Int. Cl. 5)
X	STN FILE SUPPLIER (KARLSR.) & CHEMICAL ABSTRACTS, vol. 115, no. 5 Columbus, Ohio, US; abstract no. 47345z, * abstract * & J. IMMUNOL. METHODS vol. 138, no. 1, 1991, pages 111 - 119 K. M. WILSON 'RAPID WHOLE BLOOD ASSAY FOR HIV-1 SEROPOSITIVITY USING AN FAB-PEPTIDE CONJUGATE' * the whole document *	1-2,6-8, 9,12	
X	DE-A-4 014 540 (TSCHAIKOWSKY K.) 14 November 1991 * page 2, line 10 - line 16; claims *	1-15	
X	WO-A-9 212 729 (CYTEL CORPORATION) 6 August 1992 * page 15, line 24 - page 16, line 2 * * page 20, line 27 - page 21, line 31; claims 1-2,11-12 *	1,6-9, 13-14	

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INCOMPLETE SEARCH

Reason : In view of the large number of compounds which are defined by the terms such as "compound" or "ligand or fragment" or "receptor of an endothelial cell,, platelet" of claims 1,7, and "antigen of a pathogen or immunologically fragment" (claim 12), "drug" (claim 13), the search was limited to the compounds mentioned in the description and examples.
(EP: Art. 84; Guidelines, Part. B, Chapt. II.7 last sentence and Chapt. III,3.7).

Remark : Although claims 1-7 are directed to a method of treatment of (diagnostic method practised on) the human/animal body (Article 52(4) EPC) the search has been carried out and based on the alleged effects of the compound/composition.

